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ISOENZYME PROFILE AS PARAMETER TO DIFFERENTIATE PATHOGENIC STRAINS OF *Entamoeba histolytica* IN BRAZIL

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SUMMARY

The isoenzyme profiles (IP) of 33 strains of *Entamoeba histolytica* isolated from patients and carriers of two regions in Brazil (Amazonia and Southeast) were determined. The enzymes phosphoglucomutase, glucose-phosphate isomerase, hexokinase and malic enzyme were considered. IP of the strains was correlated with culture conditions, time of maintenance in laboratory and clinical history of patients. The strains were maintained under polyxenic, monoxenic and axenic culture conditions: 27 polyxenic, 1 polyxenic and monoxenic, 1 polyxenic, monoxenic and axenic and 4 axenic only. The patients were symptomatic and asymptomatic. The symptomatic patients presented either non dysenteric (NDC) or dysenteric colitis (DC), associated or not with hepatic abscess (HA). One patient presented anal amoeboma (AM). The analysis of IP for isolates maintained in polyxenic culture showed non pathogenic IP (I) for strains from carriers and patients with NDC, while the strains isolated from patients presenting DC, HA and AM resulted in isolates II or XIX pathogenic IP. This parameter was not able to differentiate strains from carriers from symptomatic patients when these strains were found in axenic or monoxenic culture. All these strains displayed pathogenic IP (II), demonstrating the inability of this parameter to classifying for virulence since it showed identical IP for strains isolated from carriers or symptomatic patients.

KEYWORDS: *Entamoeba histolytica*; Isoenzymatic profiles; Virulence; Diagnostic.

INTRODUCTION

Amoebiasis is human intestinal infection caused by *Entamoeba histolytica*, with a worldwide distribution and affecting about one-tenth of the world's population[27,29].

Asymptomatic intestinal amoebiasis is known as the luminal form and symptomatic intestinal form with non dysenteric (NDC) or dysenteric colitis (DC) with diarrhea or dysenteric and other different forms of the disease. *E. histolytica* can cause hepatic abscess (HA) or other form of extraintestinal amoebiasis due to migration of amoebae to several body regions[5,6,28].

Clear differences in the behavior of *E. histolytica* related or not to human disease are based in the existence of pathogenic and non pathogenic strains, confirmed by biological tests[13,25], monoclonal antibodies[23], isoenzymatic patterns[19,20] and DNA probes[4,24].

Isoenzymatic patterns were widely used in the 80s and early 90[2,8,15,16] to separate pathogenic from non pathogenic strains and to delineate the distribution of *E. histolytica* zymodemes in several regions of the world[8,17].

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In Brazil asymptomatic and NDC forms of the amoebiasis are common, whereas DC is scarce and cases of HA are rare[6].

SILVA et al.[22] studying five strains of *E. histolytica* cultured under axenic growth conditions found no correlation between the IP and the clinical form of the patients. NOZAKI et al.[12] examined the IP of 54 *E. histolytica* strains from Amazonian children and 42 strains that were collected from Northeast region individuals and cultured under polyxenic growth conditions; 9.3% of the Amazonian strains presented pathogenic pattern (Zymodeme XIX) and all the Northeast strains showed non pathogenic pattern.

In an effort to know the IP of Brazilian strains as a parameter to differentiate pathogenic and non pathogenic strains of *E. histolytica*, we studied 33 strains isolated from two regions of Brazil between 1972 and 1992.

MATERIAL AND METHODS

E. histolytica strains

Thirty-three strains of *E. histolytica* were isolated between 1972 and 1992, from patients presenting different clinical forms of amoebiasis. Twenty-nine strains were maintained polyxenically in Pavlova's medium modified by SILVA[21]. From these strains, twelve were transferred to ROBINSON'S[14] medium and maintained for six passages before to be used for isoenzyme electrophoresis. Two of 29 strains were also cultured in monoxenic medium with *Crithidia fasciculata* being one these 2 strains cultured axenically in TPS-1 and TYI-S-33 medium. Four strains, one isolated from patient with dysenteric colitis and three from asymptomatic carriers were cultured only axenically.

Clinical history of patients

Full clinical records of all patients were obtained. It was observed general symptoms; physical examination; rectosigmoidoscopy to observe possible lesions in the intestinal mucosa and rectal biopsy to detect trophozoites. After diagnosis all patients were treated.

Isoenzyme pattern (IP)

The lysates were obtained as described by SARGEAUNT & WILLIAMS[15]. The following enzymes were studied: [E.C.1.1.1.40] L malate: NADP+ oxidoreductase (malic enzyme) (ME); [E.C.5.3.1.9] glucose phosphate isomerase (GPI); [E.C.2.7.5.1] phosphoglucomutase (PGM) and [E.C.2.7.1.1] hexokinase (HK). The IP was determined by horizontal thin layer starch gel electrophoresis, at 4°C, according to SARGEAUNT & WILLIAMS[15,16] for PGM, GPI and ME and according to FARRI et al.[7] for HK. As reference patterns, the zymodeme II[18] pathogenic of the strains HM1-IMSS, HK-9 and NIH-200 were used. The IP of *C. fasciculata* and a pool of bacteria from the original bacterial flora from patients and asymptomatic carriers were also determined, with the aim of distinguishing these bands from the amoebae bands.

RESULTS

The IP analysis of the 33 *E. histolytica* strains isolated from patients from the Amazonian and Southeast regions of Brazil showed 3 of the 20 zymodemes reported by SARGEAUNT[18] for enzymes PGM, GPI, ME and HK, the zymodeme I, characteristic of non pathogenic strains and zymodeme II and XIX characteristic of pathogenic strains. The Figure 1 shows a photograph and a diagrammatic representation of the IP found for each strain of *E. histolytica*. The IP of bacteria and *C. fasciculata* controls are also shown.

Twenty-nine of the 33 strains studied were maintained under polyxenic growth conditions in Pavlova's and/or in Robinson's medium. Seventeen of these strains isolated from asymptomatic carriers, showed non pathogenic IP (zymodeme I). Two other strains isolated from symptomatic patients one with DC and HA and the other with anal amoeboma showed pathogenic IP (zymodeme XIX and II respectively). The 10 strains isolated from patients with NDC showed non pathogenic IP (zymodeme I). The strains cultured either in axenic or monoxenic (with *C. fasciculata*) medium showed pathogenic IP independent of the clinical history of the patients (Table 1).

DISCUSSION

Several authors have used the IP to differentiate pathogenic and non pathogenic strains of *E. histolytica*[3,19,20]. However, other investigators[1,10,11,26] found that strains with non pathogenic IP changed to pathogenic IP after axenic culture, and virulence also changed.

Polyxenic cultures in which amoebae are associated with the bacterial flora, maintain a non pathogenic IP of the asymptomatic clinical form. A similar correlation was observed between symptomatic patients with DC, HA and/or AM, and pathogenic IP. Nevertheless, no correlation was found between strains isolated from symptomatic patients with NDC and non pathogenic zymodeme I. This might be explained by the fact of these strains of *E. histolytica* present low virulence and probably be non invasive only causing erosions of the colonic mucosa, showing well determined characteristics different those of non virulent strains[6,28]. On the other hands, as suggested by CLARK & DIAMOND[3] these patients

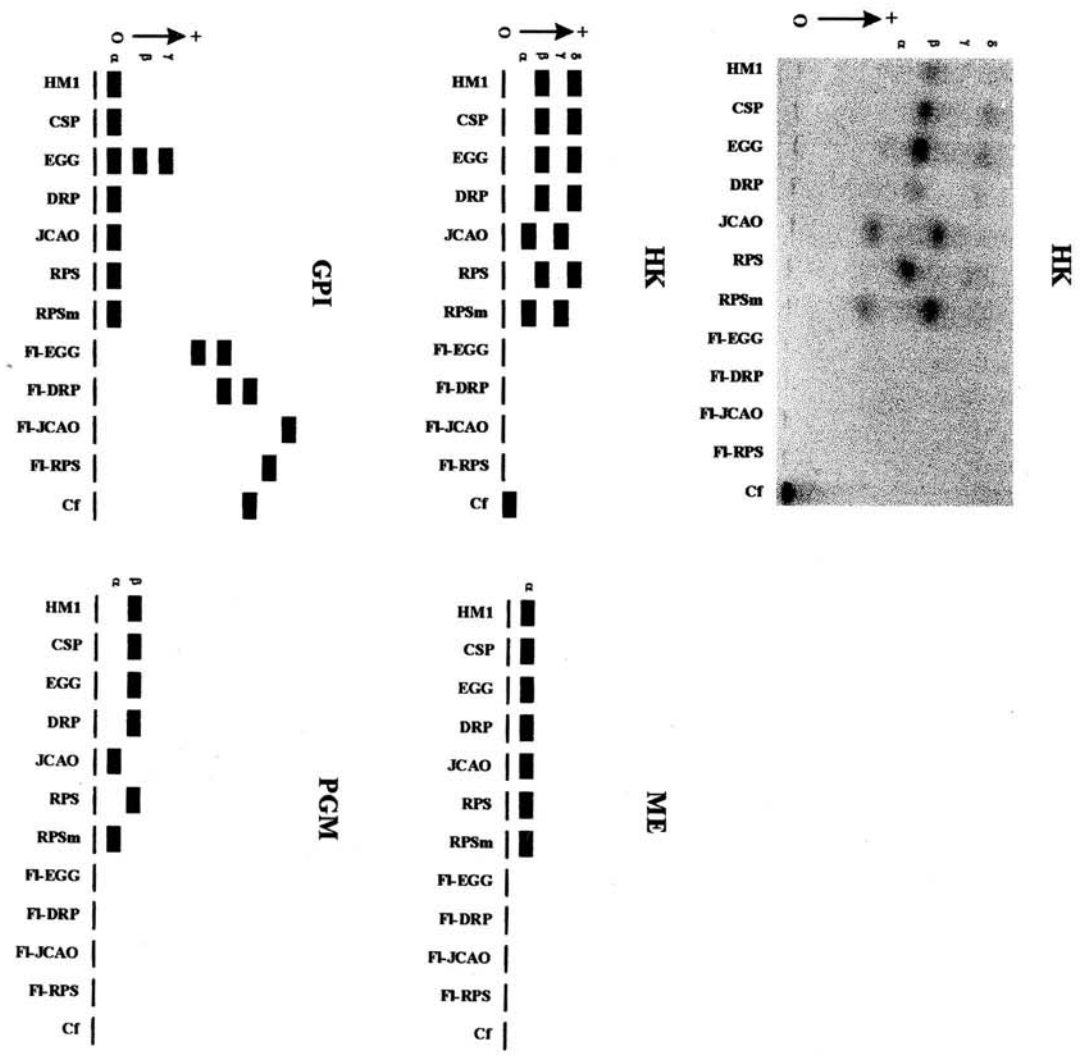

Fig. 1 – Isoenzyme pattern (IP) of HK of different *Entamoeba histolytica* strains and diagrammatic representation of IP of HK, ME, GPI and PGM. The strains HM1, CSP, EGG, DRP and RPS are characteristics of pathogenic isolates and the strains RPSm and JCAO characteristics of non pathogenic isolates. F1. is the bacterial flora of the respective *E. histolytica* strains and CF is *Crithidia fasciculata* used as controls.

with NDC, presenting zymodeme I, could be infected by *E. dispar*, the avirulent pathogen morphologically similar to *E. histolytica*. In this context we believe that NDC etiopathogeny need to be well evaluated for virulence in animal models, including a large number of strains isolated from patients with well defined clinical history and serology before determination of zymodemes for we can certainly define the etiological agent. Nevertheless our results showing the IP as not a good method for determine treatment, since it showed as non pathogenic the strains isolated from symptomatic NDC patients. The NDC clinical form is the more prevalent in Brazil (39.38%) and need adequate therapeutic treatment.

As observed by other investigators[1-10], all strains maintained in axenic or monoxenic conditions isolated from symptomatic or asymptomatic patients showed pathogenic IP. These results were observed for strains

TABLE 1

Izoenzymatic pattern from strains of *Entamoeba histolytica* cultured in axenic and polyxenic conditions, correlation with the culture medium, time of maintenance in the laboratory and the clinical history of patients.

Strains	Clinical form	I.P.	MT (years)	Culture medium
113	Sympt. (NDC)	I	13	Pavlova
99	Asympt.	I	13	Pavlova
109	Asympt.	I	13	Pavlova
177	Asympt.	I	13	Pavlova
05	Asympt.	I	8	Pavlova, Robinson
78	Sympt. (NDC)	I	8	Pavlova, Robinson
244*	Sympt. (NDC)	I	8	Pavlova
DRP*	Sympt. (amoeboma)	II	7	Pavlova, Robinson
EEM*	Asympt.	I	4	Pavlova, Robinson
MCR*	Asympt.	I	4	Pavlova
15AM*	Asympt.	I	4	Pavlova, Robinson
AL*	Asympt.	I	4	Pavlova
MC*	Asympt.	I	4	Pavlova
MF*	Asympt.	I	4	Pavlova
EGG*	Sympt. (DC, HA)	XIX XIX	4	Pavlova, Robinson monoxenic (TPS-1 + *C. fasciculata*)
VEJ	Sympt. (NDC)	I	4	Pavlova
SPS	Sympt. (NDC)	I	3	Pavlova
JN*	Asympt.	I	3	Pavlova
SC*	Asympt.	I	3	Pavlova, Robinson
RPS*	Asympt.	I II	3	Pavlova, Robinson axenic and monoxenic (TPS-1 + *C. fasciculata*)
LMM*	Sympt. (NDC)	I	3	Pavlova
JCAO*	Sympt. (NDC)	I	3	Pavlova, Robinson
PCQ*	Asympt.	I	3	Pavlova
MLES*	Asympt.	I	3	Pavlova
EDI	Asympt.	I	1.5	Pavlova, Robinson
NB	Asympt.	I	1.5	Pavlova
IA	Sympt. (NDC)	I	0.8	Pavlova, Robinson
MM	Sympt. (NDC)	I	0.8	Pavlova, Robinson
MA	Sympt. (NDC)	I	0.8	Pavlova
CSP	Sympt. (DC)	II	9	axenic (TPS-1, TYI-S-33)
32	Asympt.	II	12	axenic (TPS-1, TYI-S-33)
462	Asympt.	II	12	axenic (TPS-1, TYI-S-33)
452	Asympt.	II	9	axenic (TPS-1, TYI-S-33)

I.P. Isoenzymatic Pattern; **MT** Maintenance Time in culture; **Sympt.** symptomatic; **Asympt.** asymptomatic; **DC** dysenteric colitis; **NDC** non dysenteric colitis: **HA** hepatic abscess.

*Strains from Amazonian region. The others are from Southeast region of Brasil.

isolated from carriers (RPS, 32, 452 and 462), which showed a type II zymodeme similar to CSP and EGG strains from symptomatic patients. However, these strains isolated from carriers never showed virulent behavior as "in vivo" as "in vitro" assay (data not shown), demonstrating the inability of this parameter to classifying for virulence since it showed identical IP for strains isolated from carriers or symptomatic patients.

RESUMO

Perfil isoenzimático como parâmetro para diferenciar cepas patogênicas de *Entamoeba histolytica* no Brasil

O perfil isoenzimático (PI) de 33 cepas de *E. histolytica*, isoladas das regiões Amazônica e Sudeste do Brasil foi determinado. Foram consideradas as enzimas fosfoglicomutase, glicose fosfato isomerase, hexoquinase e enzima málica. O perfil obtido para cada cepa foi correlacionado com o meio e o tempo de manutenção em cultivo e com a história clínica do paciente. As cepas foram mantidas sob condições de cultivo axênico, monoxênico e polixênico: 27 polixênico, 1 polixênico e monoxênico, 1 polixênico, monoxênico e axênico e 4 somente em cultivo axênico. Os pacientes apresentaram sintomas ou não. Os pacientes sintomáticos apresentaram colite disentérica (CD) ou colite não disentérica (CND), associada ou não com abscesso hepático (AH). Um paciente apresentou ameboma anal (AM). A análise do PI das diferentes cepas mantidas em cultivo polixênico mostrou um PI não patogênico (I) para as cepas isoladas de portadores e pacientes com CND, enquanto as cepas isoladas de pacientes apresentando CD, AH ou AM resultou em isolados com PI patogênico (II e XIX). Analisando o PI das cepas mantidas em cultura axênica ou monoxênica, verificamos a inabilidade deste parâmetro para diferenciar cepas isoladas de portadores daquelas isoladas de pacientes sintomáticos. Todas estas cepas apresentaram PI patogênico, demonstrando a inabilidade deste parâmetro para classificar quanto à virulência.

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